Elimination and Reconstitution of the Requirement for Hormone in Promoting Temperature-dependent Transformation of Cytosolic Glucocorticoid Receptors to the DNA-binding State*

(Received for publication, September 18, 1989)

Soheil Meshinchi, Edwin R. Sanchez, Karen J. Martell, and William B. Pratt‡

From the Department of Pharmacology, The University of Michigan Medical School, Ann Arbor, Michigan 48109

Cytosols contain a heat-stable, chelatable, anionic, molybdate-like factor that stabilizes glucocorticoid receptors in a heteromeric complex with hsp90 (refers to the 90-kDa heat shock protein) and inhibits their transformation to the DNA-binding state (Meshinchi, S., Grippo, J. F., Sanchez, E. R., Bresnick, E. H., and Pratt, W. B. (1988) J. Biol. Chem. 263, 16809-16817). In this way, we demonstrate that removal of this factor by passage of L cell cytosol through the metal-chelating resin Chelex-100 makes the glucocorticoid receptor unstable, thus markedly facilitating both its dissociation from hsp90 and its transformation to the DNA-binding state. In normal cytosol, both temperature-mediated dissociation of hsp90 and temperature-mediated receptor transformation are hormone-dependent events. In the Chelex-treated, metal-depleted cytosol, however, temperature-mediated dissociation of hsp90 and receptor transformation oc

In 1977, our laboratory published that a heat-stable system in L cell cytosol was capable of converting glucocorticoid receptors from a nonsteroid-binding to a steroid-binding form (1). The heat-stable system was found to contain two activities (2, 3); a receptor reducing factor that was subsequently identified as thioredoxin (4, 5), and a small receptor stabilizing factor (3). We recently showed that the endogenous metal anion interacts with the glucocorticoid receptor as an endogenous inhibitor of glucocorticoid receptor transformation reported by the laboratories of Litwack et al. (7, 8) and Milgrom et al. (9) and subsequently shown by Sato et al. (10) to inhibit the transformation of androgen and estrogen receptors. The factor behaves as a strong anion with an apparent M, of 340 on Bio-Gel P-2, it is stable to heating at 320 °C for 1 h, and it binds tightly to Chelex-100, a metal chelating resin (6). Thus, we have proposed that the factor is a metal anion and that molybdate and vanadate may exert their effects on the glucocorticoid receptor by interacting with the binding site for this endogenous metal anion. Bodine and Litwack (11, 12) have also published the purification of a cytosol factor with the same molybdate-like actions on glucocorticoid receptors but have proposed an organic composition of a phosphoglyceride nature.

If this endogenous factor plays a role in stabilizing cytosolic glucocorticoid receptors in their untransformed complex with hsp90, then removal of the factor from cytosol should result in complexes that are less stable and more readily transformed to their DNA-binding state. In this paper we show that removal of endogenous metals from L cell cytosol with Chelex-100 resin facilitates both dissociation of the glucocorticoid receptor from hsp90 and conversion of the glucocorticoid receptor to the DNA binding state. It has previously been shown that glucocorticoid receptor transformation in normal cytosol preparations is both temperature-dependent and hormone-dependent (13, 14). In contrast, in metal-free cytosol, glucocorticoid receptor dissociation from hsp90 and transformation to the DNA-binding state are still temperature-dependent events but they are no longer hormone-dependent. We show that readdition of heat-stable components of L cell cytosol to this system reconstitutes the requirement for hormone for promoting both dissociation of hsp90 and generation of the DNA-binding state. To our knowledge, this is the first

* The term transformation will be used throughout this paper to describe the process whereby the receptor is converted from a non-DNA-binding to a DNA-binding form.

‡ To whom correspondence should be addressed.
time that the requirement for the ligand has been eliminated and reconstituted in a cell-free system in which receptors are converted from an inactive to an active conformation.

**EXPERIMENTAL PROCEDURES**

**Materials**

[6,7-3H]Triamcinolone acetonide (42.8 Ci/mmol), and 125I-conjugated goat anti-mouse IgG were obtained from Du Pont-New England Nuclear. Radioimnunodexamethasone, rabbit anti-goat IgG-horseradish peroxidase conjugate, sodium molybdate, Hepes buffer, and marker proteins were from Sigma. Chelex-100 chelating ion exchange resin and chemicals for electrophoresis were from Bio-Rad. Immobilon-P transfer membrane (0.45 µm) was from Millipore (Bedford, MA). The BuGR2 anti-glucocorticoid receptor monoclonal antibody was kindly provided by Dr. R. W. Harrison (University of Arkansas) (15), and the AC88 monoclonal antibody against the 90-kDa heat shock protein (16) was kindly provided by Dr. David Toft (Mayo Medical School).

**Methods**

**Cell Source and Fractionation**—L929 murine fibroblasts were grown in monolayer culture in Dulbecco’s modified eagle medium supplemented with 10% calf serum at 37 °C. Cells were harvested by scraping and washed in Earle’s-balanced saline. The cell pellet was suspended in 1.5 volumes of 10 mM Hepes, 0.1 mM EDTA, pH 7.4 at 4 °C, and ruptured by Dounce homogenization. The homogenate was centrifuged at 100,000 × g. The supernatant (referred to as cytosol) was used immediately.

**Preparation of Stabilizing Factor**—Stabilizing factor was prepared by boiling rat liver cytosol, centrifuging out the denatured material, and passing the supernatant through sequential columns of Sephadex G-50 and G-10 as described previously (6). The factor preparation was concentrated 16-fold by lyophilization, and the concentration of factor is expressed in relative units, with one relative unit of factor being the concentration of factor normally present in L cell cytosol (6).

**Chelex Treatment of Cytosol**—Chelex resin was washed extensively and equilibrated with 10 mM Hepes buffer, pH 7.2 or 8.2 as noted in the figure legends. The resin was loaded into a 10-ml syringe and centrifuged inside a 30-ml Corex tube at 10,000 × g in order to remove buffer from within the beads. L cell cytosol containing either 3H-stereoid-bound or unliganded receptors was then adjusted to pH 7.2 or 8.5, placed on top of the resin in a 1:4 ratio (cytosol volume/resin volume), and centrifuged through the resin itself. This method allows for removal of Chelex-bound metals from cytosol without dilution of the receptor and with minimum loss of steroid binding activity. It is important to emphasize that in order to have appropriately low zero time values of receptor transformation, the experiments should be carried out with freshly prepared cytosol, treatment with Chelex and separation from Chelex resin must be performed as rapidly as possible, samples must be kept rigorously cold prior to subsequent procedures, and it is important not to dilute the Chelex-treated cytosol.

**Incubation Conditions**—Samples of whole (untreated) cytosol or Chelex-treated cytosol were incubated at the temperatures noted in the figure legends. In experiments with steroid-bound receptors, samples of whole or Chelex-treated cytosol were incubated for 1.5 h on ice with 600 nM nonradioactive dexamethasone prior to incubation at 15 or 20 °C. At various times, aliquots were removed, and sodium molybdate was added to a final concentration of 50 mM to stop subsequent dissociation of hsp90 and transformation to the DNA-binding state. Steroid binding and DNA binding activity of the glucocorticoid receptor were assayed as described below. Receptor-associated hsp90 was assayed by immunoadsorbing the glucocorticoid receptor from each sample onto protein-A-Sepharose, resolving the immunoadsorbed proteins by SDS-PAGE, and quantitating the amount of hsp90 by probing an immunoblot with the AC88 monoclonal antibody against hsp90. The relative amount of glucocorticoid receptor-associated hsp90 in each sample was determined by reacting the immunoblot with a 125I-labeled antibody directed against mouse IgG as described previously (17).

**Receptor Immunoadsorption**—After addition of molybdate, aliquots of cytosol (100 µl for assay of receptor; 400 µl for assay of receptor-associated hsp90) were mixed with an equal volume of TEGM buffer (10 mM TES, 4 mM EDTA, 10% glycerol, 50 mM NaCl, 20 mM sodium molybdate, pH 7.6 at 4 °C). BoGR antireceptor antibody was added at 2% of the final volume, the mixture was incubated for 2 h at 0 °C, and each sample (20 µl) was then added to a protein-A-Sepharose pellet (10 µl pellet/0.1 ml of L cell cytosol). Samples were mixed by rotation for 3 h at 4 °C and protein-A-Sepharose pellets were washed three times by reconstitution in 1 ml aliquots of TEGM buffer. Both glucocorticoid receptor and receptor-associated hsp90 were assayed by SDS-PAGE and immunoblotting.

**Assay of Steroid Binding Capacity and DNA Binding Activity of the Glucocorticoid Receptor**—Steroid binding capacity was assayed by incubating aliquots (45 µl) of various samples (as described in Table 1 and Fig. 1) for 8 h in 1 mM buffer, pH 7.2, containing 50 mM [3H]triamcinolone acetonide in the presence or absence of competing cold dexamethasone. Each incubation mixture was then mixed with 0.15 ml of a suspension of dextran-coated charcoal (1% charcoal (w/v) and 0.2% dextran (w/v) in 10 mM Hepes, pH 7.3) for 10 min at 0 °C. The radioactivity remaining in the charcoal supernatant was assayed and the specific binding was determined by subtracting radioactivity obtained in the presence of cold dexamethasone from that obtained in its absence.

To assay DNA-binding capacity, aliquots of receptor preparation were mixed with a 12.5% DNA-cellulose suspension in 10 mM Hepes, pH 7.3, containing 30 mM sodium molybdate, and stirred on ice for 45 min. DNA-cellulose pellets were washed three times in 10 mM Hepes buffer. As described in the figure legends, DNA-cellulose pellets were either subjected to scintillation counting for assay of DNA-bound [3H]TAM receptor complexes, or the pellets were extracted with SDS-sample buffer and DNA-bound receptor was assayed by quantitative Western immunoblot analysis with a 125I-labeled second antibody probe as described above.

**Preparation of Boiled Cytosol**—L cell cytosol was boiled for 15 min in a boiling water bath, and centrifuged at 12,000 × g for 5 min. The supernatant was collected and lyophilized to a 20-fold concentrated stock solution. To prepare metal-free boiled cytosol, 0.25 ml of 20-fold concentrated boiled cytosol was loaded onto a column of Chelex-100 resin (1.5 × 10 cm) which was equilibrated at pH 7.2 with 10 mM Hepes buffer. The column was subsequently eluted with 10 mM Hepes buffer, pH 7.2, and the dropthrough fractions were collected, lyophilized to dryness, and reconstituted to the original volume in 10 mM Hepes buffer, pH 7.2.

**RESULTS**

**Passage of Cytosol through Chelex-100 Promotes Transformation of the Glucocorticoid Receptor**—In our previous publication (6), we showed that passage of boiled cytosol through Chelex-100 resin removes endogenous metals, such as molybdenum, zinc, and aluminum and also removes the endogenous receptor stabilizing activity. Fig. 1 shows that Chelex-treatment of whole cytosol promotes glucocorticoid receptor transformation. In the first several experiments shown in this paper, transformation will be brought about by elevating the cytosolic pH as originally described by Baille et al. (19). Although it is not clear why elevating the pH promotes transformation, it is a useful way to facilitate the process at 0 °C without exposing the receptor complex to high concentrations of salt or diluting the cytosol. As shown in Fig. 1, under conditions where less than 20% of receptors in untreated cytosol are transformed to the DNA-binding state at
Alkaline pH-mediated transformation of glucocorticoid receptor in Chelex-treated cytosol is accompanied by a change in the size of the receptor complex as reflected by a reduction in the sedimentation coefficient from 9 S to 4 S, and this reduction in apparent receptor size is also inhibited by either the stabilizing factor or molybdate (not shown).

There is considerable evidence that both glucocorticoid receptor transformation to the DNA-binding state and its conversion from the 9 S to 4 S form is due to the dissociation of the receptor from hsp90 (see Refs. 20 and 21 for review). In the experiment shown in Fig. 2, we show that receptors in Chelex-treated, pH 8.2 cytosol dissociate from hsp90 at 0 °C. To interpret this data it is important to know (as documented later in Fig. 5) that Chelex treatment does not alter the ability of the receptor to be recognized by the BuGR antibody. Both sodium molybdate (lane 4) and the heat stable factor (lane 5) are able to inhibit the alkaline pH-mediated loss of hsp90 from the Chelex-treated receptor complexes. Thus, it appears that removal of the endogenous stabilizing factor by Chelex treatment of cytosol facilitates both dissociation of the receptor from hsp90 and generation of the DNA-binding state caused by elevation of pH.

Temperature-dependent Transformation of GR in Chelex-treated Cytosol Is No Longer Steroid-dependent—When L cell cytosol containing hormone-free glucocorticoid receptor is incubated at 25 °C, the receptors lose their ability to bind steroid; a similar incubation of steroid-bound glucocorticoid receptor converts receptors to the DNA-binding state (22). The experiment of Fig. 3 presents the rates of inactivation of steroid binding capacity of unliganded receptors (panel A) and the rates of transformation of steroid-bound receptors (panel B) in both Chelex-treated and untreated cytosol. It is clear that the rate of temperature-dependent inactivation of steroid binding capacity and the rate of transformation to the DNA-binding state are more rapid in the metal-depleted, Chelex-treated cytosol than in whole cytosol. In Chelex-treated cytosol, both glucocorticoid receptor inactivation and transformation proceed very rapidly at 25 °C and the experiments of this paper are carried out at 15 or 20 °C to slow down both events.

In our usual L cell cytosol preparations containing all of the endogenous metals, binding of glucocorticoid to the receptor promotes temperature-dependent transformation of the receptor to the DNA binding form (13). To determine if steroid was still required after removal of metals, we performed the experiments of Fig. 4. The figure presents the transformation of both steroid-bound and unbound receptors...
FIG. 3. Chelex treatment of cytosol promotes temperature-mediated glucocorticoid receptor inactivation and transformation. Panel A, inactivation of steroid binding capacity. Either untreated cytosol or cytosol passed through Chelex resin at pH 7.2 was incubated at 0 or 15 °C. At various times, aliquots were removed and the steroid binding capacity was determined by binding with [3H]triamcinolone acetonide. The data are presented as the percent of zero time binding capacity. Panel B, receptor transformation. Either untreated cytosol or pH 7.2 Chelex-treated cytosol containing steroid-bound receptors was incubated at 0 or 15 °C. At various times, aliquots were removed for determination of both steroid and DNA binding. Data are presented as the percent of total receptor bound to DNA-cellulose. In both panels the values represent the mean and standard error of determinations from three separate experiments. Where no error bars are shown, the error lies within the symbols. The conditions in this and following figures are indicated as follows: R, steroid-free receptor in untreated cytosol; RS, steroid-bound receptor in untreated cytosol; CR, steroid-free receptor in Chelex-treated cytosol; CRS, steroid-bound receptor in Chelex-treated cytosol. Samples are O, untreated cytosol incubated at 0 °C; ©, untreated cytosol incubated at 15 °C; A, Chelex-treated cytosol incubated at 0 °C; A, Chelex-treated cytosol incubated at 15 °C.

FIG. 4. Temperature-mediated transformation of unliganded and steroid-bound receptors to the DNA-binding state. Whole cytosol was divided into two portions, one of which was passed through Chelex resin (pH 7.2). Half of each portion was then incubated 2 h at 0 °C with 100 nM nonradioactive dexamethasone to form steroid-bound receptors. Portions of each condition were then incubated at 0 or 15 °C, and at various times, aliquots were removed and incubated with DNA-cellulose at 0 °C. The amount of DNA-bound receptor was assayed by the quantitative Western immunoblot procedure using a 125I-labeled second antibody probe as described under “Materials and Methods.” Each value in the diagram represents the mean and standard error of determinations from three separate experiments. Autoradiograms of Western blots of glucocorticoid receptor from one experiment are presented above the diagrams. To obtain the values plotted in the diagram each band on the Immobilon transfer membrane was excised and counted. To permit normalization of values from different experiments, the amount of DNA-bound receptor is expressed in both panels as a percent of the highest DNA-binding value achieved in Chelex-treated cytosol. Panel A represents values from the Chelex-treated portion of cytosol and panel B from the untreated whole cytosol. ©, unliganded (steroid-free) receptor in whole (R) or Chelex-treated (CR) cytosol at 0 °C; ©, unliganded receptor in whole or Chelex-treated cytosol at 15 °C; ©, steroid-bound receptor in whole (RS) or Chelex-treated (CSR) cytosol at 0 °C; ©, steroid-bound receptor in whole or Chelex-treated cytosol at 15 °C.
Each sample was incubated at 20 °C and at various times aliquots were bound with nonradioactive dexamethasone anti-receptor antibody. The immunoadsorbed proteins were extracted and receptors were immunoadsorbed with the BuGR Chelex-treated (pH 7.2) cytosol were divided into two portions and receptor-associated hsp90 in each sample is presented as a percent of the zero time value. The amount of receptor in each condition was also quantitated by probing the Western blots with BuGR and unliganded and steroid-bound receptors.

As shown in panel A of Fig. 4, Chelex-treated cytosol is rapidly transformed to the DNA-binding state in a temperature-dependent manner regardless of whether it is steroid-bound or not. At 0 °C, neither liganded nor unliganded Chelex-treated cytosol is transformed to the DNA-binding state. In the case of whole cytosol (i.e. non-Chelex-treated), the steroid-bound receptor is transformed to the DNA-binding state but at a much slower rate than in Chelex-treated cytosol. Under identical conditions, unliganded receptor in Chelex-treated cytosol remains intact over the course of several hours. (data not shown).

If dissociation of hsp90 is associated with transformation of the glucocorticoid receptor to the DNA-binding state, then the rates of the two processes should be similar. The experiment shown in Fig. 6 compares the rates of both hsp90 dissociation and transformation in the same samples of Chelex-treated and whole cytosol incubated at 20 °C. Both transformation and hsp90 dissociation occur at a similar rapid rate in metal-depleted cytosol and at the same slow rate in whole cytosol.

Reconstitution of the Hormone Requirement for Temperature-dependent Dissociation of hsp90 and Transformation—

The above experiments suggest that elimination of a metal component of cytosol by chelation removes the requirement for hormone in promoting thermal dissociation of hsp90 from the receptor. In the experiment of Fig. 7, boiled L cell cytosol was added back to Chelex-treated cytosol to see if the requirement for the hormone could be reconstituted. Boiled L cell cytosol...
Glucocorticoid Receptor Transformation

The presence of the indicated combinations of buffer, one relative unit of boiled cytosol, CR g cvtosol incubated with steroid. CRS: 0. Chelex-treated cytosol incubated with buffer, steroid-free whole cytosol, R; 0, steroid-bound whole cytosol, RS; A, hsp90 antibody as the probing agent. The amount of receptor-specific antibody. Proteins were extracted from the pellet and resolved by SDS-PAGE. The amount of receptor-specific hsp90 was quantitated by _35_1 Western immunoblot analysis using AC88 monoclonal antisum of boiled L cell cytosol (the concentration of heat-stable components normally present in whole cytosol), Chelex-treated-boiled cytosol, and nonradioactive dexamethasone. This preincubation time ensures occupancy of all steroid-binding sites. Samples were then incubated at 20 °C and at various times aliquots (400 μl) were removed and immunoadsorbed to protein A-Sepharose with BuGR2 anti-receptor antibody. Proteins were extracted from the pellet and resolved by SDS-PAGE. The amount of receptor-specific hsp90 was quantitated by _35_1 Western immunoblot analysis using AC88 monoclonal antibody. The amount of receptor-specific hsp90 in each sample is presented as the % of the time zero value. O, steroid-free whole cytosol, R; steroid-bound whole cytosol, RS; A, Chelex-treated cytosol incubated with buffer, CR; A, Chelex-treated cytosol incubated with steroid, CRS; O, Chelex-treated cytosol incubated with boiled cytosol, CR + f; A, Chelex-treated cytosol incubated with boiled cytosol and steroid, CR + f + S; O, Chelex-treated cytosol incubated with Chelex-treated boiled cytosol, CR + Cf.

cytosol, which is indicated as f (for heat-stable factor preparation) in the figures, contains the endogenous receptor stabilizing factor (2, 3, 6), and as shown in Fig. 7, it inhibits thermal dissociation of hsp90. Chelex-treated boiled cytosol does not inhibit dissociation, suggesting that a metal component of boiled cytosol is responsible for stabilization of the glucocorticoid receptor-hsp90 complex. Most importantly, after readdition of the heat-stable components of cytosol to the Chelex-treated system, binding of the hormone is again required to promote dissociation of the receptor from hsp90.

The experiments of Fig. 8 demonstrate that the hormone requirement for receptor transformation to the DNA-binding state is also reconstituted when the heat-stable components of cytosol are added back to the system. In these experiments, the highest value of transformation is achieved by unbound receptors in Chelex-treated cytosol at 60 min, and this value has been set as 100% DNA binding in Fig. 8 to permit normalization of data from separate experiments. It is clear that addition of the heat-stable components of cytosol to the Chelex-treated system inhibits transformation and that dexamethasone again promotes transformation in the reconstituted system.

FIG. 7. Reconstitution of hormone requirement for the thermal dissociation of hsp90 from the glucocorticoid receptor. Aliquots of Chelex-treated cytosol were incubated for 2.5 h on ice in the presence of the indicated combinations of buffer, one relative unit of boiled L cell cytosol (the concentration of heat-stable components normally present in whole cytosol), Chelex-treated-boiled cytosol, and nonradioactive dexamethasone. This preincubation time ensures occupancy of all steroid-binding sites. Samples were then incubated at 20 °C and at various times aliquots (400 μl) were removed and immunoadsorbed to protein A-Sepharose with BuGR2 anti-receptor antibody. Proteins were extracted from the pellet and resolved by SDS-PAGE. The amount of receptor-specific hsp90 was quantitated by _35_1 Western immunoblot analysis using AC88 monoclonal antibody. The amount of receptor-specific hsp90 in each sample is presented as the % of the time zero value. O, steroid-free whole cytosol, R; steroid-bound whole cytosol, RS; A, Chelex-treated cytosol incubated with buffer, CR; A, Chelex-treated cytosol incubated with steroid, CRS; O, Chelex-treated cytosol incubated with boiled cytosol, CR + f; A, Chelex-treated cytosol incubated with boiled cytosol and steroid, CR + f + S; O, Chelex-treated cytosol incubated with Chelex-treated boiled cytosol, CR + Cf.

DISCUSSION

Because a number of steroid hormone receptors are associated with hsp90 when they are in their non-DNA-binding form, but not after conversion to their DNA-binding form, the concept has arisen that dissociation from hsp90 is required to derepress the DNA binding function of the receptor (13, 14, 23–27). This could occur either via a simple unmasking of the DNA-binding site or more likely via a major conformational change in the receptor that occurs when hsp90 dissociates (17, 28). The site of association of the glucocorticoid receptor with hsp90 and the site(s) of interaction with the metal oxyanions (e.g. molybdate, vanadate) that stabilize the receptor-hsp90 association lie within the steroid-binding domain of the receptor (29, 30). A number of studies have shown that the steroid-binding domain acts as a “regulatory cassette” in the sense that it can endow the property of glucocorticoid regulation (e.g. 28). These types of observations have led to the model that steroid occupancy of the receptor somehow promotes dissociation of the glucocorticoid receptor from hsp90 as the initial hormone event (see Refs. 20, 21 for reviews).

It is important to realize that this notion that steroid-mediated dissociation of the receptor complex is the initiating event in the hormone action is a working model. It is not known if the steroid acts only as a trigger and subsequent events are a consequence of this dissociation (20) or if the receptor must be bound with steroid for subsequent events in transcriptional activation to occur. The model already has a major exception. The receptor for thyroid hormone is a member of the same receptor family (31) and it differs from glucocorticoid, progesterone, and most other steroid receptors in that it is not recovered in the cytosolic fraction because it is very tightly associated with nuclear-binding sites prior to hormone binding. In contrast to the glucocorticoid receptor, where newly translated receptor is already bound to hsp90 and is in a non-DNA binding form (32, 33), the receptor for triiodothyronine is translated in a DNA binding form and is not bound to hsp90. In the case of the thyroid hormone receptor, and perhaps also in the case of the retinoic acid receptor, it would seem that there must be a hormone-dependent event, probably at the level of the regulated genes, that does not depend upon dissociation of a receptor-hsp90 complex.

Despite the uncertainty regarding the place of the dissociation model in steroid hormone action in the intact cell, we can certainly learn much that is of importance to a fundamental understanding of the information transduction mechanism if we can study and control rapid hormone-dependent events under cell-free conditions. Several laboratories have set up conditions, such as those used here for whole L cell cytosol, in which both dissociation of the heteromeric glucocorticoid receptor complex and transformation of the receptor to the DNA-binding state occur in a manner that requires both the presence of hormone and elevated temperature (e.g. 13, 14). It is known that glucocorticoid receptors in intact cells also acquire DNA binding activity if they are exposed to steroid at elevated temperatures but not if cells are kept at 0 °C (e.g. 34, 35). Hormone-dependent, temperature-dependent transformation of cytosolic receptors to the DNA-binding state has been used as a cell-free model of the initial event in glucocorticoid action. It is of course inherent to the concept

3 F. C. Dalman, R. J. Koenig, G. H. Perdew, and W. B. Pratt, manuscript submitted.
The impression that the receptor is converted from a biologically inactive to an active state when it acquires DNA binding activity drives both dissociation of the receptor complex and transformation. This leads us to think that another heat stable component of cytosol is required for reconstitution of hormone dependence in addition to the metal that is removed by Chelex.

Transformation of cytosolic glucocorticoid receptors can be brought about without the presence of hormone by diluting cytosol or by increasing the pH or the ionic strength. These are conditions that also promote dissociation of the receptor from hsp90, and they may serve to counteract the stabilizing effect of the endogenous metal anion on the receptor-hsp90 complex. Transformation can also be brought about by heating cytosol. When the temperature of L cell cytosol is raised as high as 37 °C, the glucocorticoid receptor rapidly dissociates from hsp90 and acquires DNA binding activity even when it is not bound by steroid (data not shown). It is only at lower temperatures where one can easily demonstrate in whole cytosol that steroid binding and thermal energy are both required for receptor transformation.

The fact that hormone-free glucocorticoid receptor in metal-depleted cytosol undergoes temperature-dependent dissociation and transformation at the same rate as hormone-bound glucocorticoid receptor (Figs. 4 and 5) suggests that there is a temperature-dependent component of transformation that is independent of the steroid-dependent component. The observations of this paper also suggest that the steroid-dependent transformation of receptors that occurs at temperatures where this event is usually measured in cytosol preparations (20–27 °C) may reflect an effect of steroid binding on the ability of the endogenous metal anion to stabilize the complex. The binding of steroid to its receptor site could affect such a metal center either directly through a conformational change that affects the positioning of the metal-binding ligands or perhaps indirectly by affecting the redox state of metal-binding ligands, such as cysteine thiol groups located in the steroid-binding domain.

A model that could explain the observations of this paper and the concept of independent temperature-dependent and ligand-dependent events assumes two principal centers of interaction between hsp90 and the glucocorticoid receptor, with only one of these sites being involved in metal binding. One site that has been suggested for hsp90 binding is a highly conserved hydrophobic region that lies within the steroid-binding domain (29, 36). This region may provide the major contribution to the binding forces that are disrupted in a temperature-dependent manner when metal is no longer present to stabilize the second site of interaction with hsp90. We know from the study of mutant mouse receptors in which this region is deleted that the metal anion stabilization site is situated outside of the conserved domain, most probably toward the carboxyl terminus of the steroid-binding domain.4

4 P. R. Housley, E. R. Sanchez, G. M. Ringold, and W. B. Pratt, manuscript in preparation.

![Figure 8](https://example.com/figure8.png)

**Fig. 8. Reconstitution of hormone requirement for glucocorticoid receptor transformation.** Aliquots of Chelex-treated cytosol or whole cytosol were preincubated at 0 °C with additions as described in the legend to Fig. 8. Samples were then heated at 15 °C, and at various times aliquots (100 µl) were removed and incubated with DNA-cellulose on ice. The DNA-bound receptor was quantitated by 125I-Western immunoblot analysis using the BuGR2 antireceptor antibody. The data are averaged from three experiments and are presented as a percent of the highest DNA-binding achieved at 60 min by receptors in steroid-free, Chelex-treated cytosol (CR). Panel A, △, DNA binding of receptors in Chelex-treated cytosol incubated with buffer; □, Chelex-treated cytosol incubated with boiled cytosol and steroid; ○, Chelex-treated cytosol incubated with Chelex-treated boiled cytosol. Panel B, ○, unliganded whole cytosol; ●, steroid-bound whole cytosol.
By examining the transcriptional activating activity of glucocorticoid receptors with carboxyl-terminal truncations and internal deletions, Hollenberg et al. (37) have provided evidence that two distinct regions within the steroid-binding domain are responsible for repression of receptor function. Again, these are results that support the possibility of two distinct protein-binding sites within the steroid-binding domain. Binding of hsp90 to the glucocorticoid receptor at two independent sites might be expected to yield a high affinity interaction that is an exponential function of the two binding affinities. Thus, by affecting only a metal anion site, the steroid could have a very large effect in promoting destabilization of the complex, as long as sufficient thermal energy is provided to promote dissociation at the second site. As suggested earlier (17), the collapse of the conserved hydrophobic region from the receptor surface when hsp90 dissociates could result in a large conformational change in the receptor with accompanying derepression of the DNA-binding function.

The metal-depleted cytosol system that we have described here has provided us with the ability to eliminate and then reconstitute the requirement for hormone in promoting temperature-dependent receptor transformation. The relationship between cytosolic receptor transformation and the initial events that lead to a hormone response in the intact cell is unclear. Indeed, the use of broken cell preparations may create artifacts not occurring in intact cells. In intact cells, the steroid receptors must interact with multiple proteins that are responsible for their transport to the nucleus, passage through nuclear pores, and finally directed transfer to high affinity “acceptor sites” providing access to genes under the control of glucocorticoid response elements. Thus, the cytosolic system of glucocorticoid receptor transformation we have studied here is obviously simplified with respect to the intact cell. But at least this cytosolic system provides us with an effect that is regulated by hormone binding to the receptor and that can be studied at the molecular level under controlled conditions. Eventually, this system may prove to be useful in helping us to differentially examine the kinds of early and admittedly very speculative events discussed above.

Acknowledgments—We would very much like to thank Drs. David O. Toft and Robert W. Harrison, III for generously providing the AC88 anti-hsp90 and BuGR2 anti-receptor monoclonal antibodies, respectively.

REFERENCES
Elimination and reconstitution of the requirement for hormone in promoting temperature-dependent transformation of cytosolic glucocorticoid receptors to the DNA-binding state.
S Meshinchi, E R Sanchez, K J Martell and W B Pratt


Access the most updated version of this article at http://www.jbc.org/content/265/9/4863

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/265/9/4863.full.html#ref-list-1